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Folate Deficiency Is Prevalent in Women of Childbearing Age in Belize and Is Negatively Affected by Coexisting Vitamin B-12 Deficiency: Belize National Micronutrient Survey 2011^{1–4}

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Abstract

Background—Folate deficiency, vitamin B-12 deficiency, and anemia can have adverse effects on birth outcomes. Also, low vitamin B-12 reduces the formation of metabolically active folate.

Objectives—We sought to establish the baseline prevalence of and factors associated with folate deficiency and insufficiency, vitamin B-12 deficiency, and anemia among women of childbearing age (WCBA) in Belize.

Methods—In 2011, a national probability-based survey was completed among Belizean nonpregnant WCBA aged 15–49 y. Blood samples for determination of hemoglobin, folate (RBC and serum), and vitamin B-12 (plasma) and sociodemographic and health information were collected from 937 women. RBC and serum folate concentrations were measured by microbiologic assay (MBA). Folate status was defined based on both the WHO-recommended radioproteinbinding assay and the assay adjusted for the MBA.

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⁴Supplemental Tables 1–4 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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Results—The national prevalence estimates for folate deficiency in WCBA, based on serum and RBC folate concentrations by using the assay-matched cutoffs, were 11.0% (95% CI: 8.6%, 14.0%) and 35.1% (95% CI: 31.3%, 39.2%), respectively. By using the assay-matched compared with the WHO-recommended cutoffs, a substantially higher prevalence of folate deficiency was observed based on serum (6.9% absolute difference) and RBC folate (28.9% absolute difference) concentrations. The prevalence for RBC folate insufficiency was 48.9% (95% CI: 44.8%, 53.1%). Prevalence estimates for vitamin B-12 deficiency and marginal deficiency and anemia were 17.2% (95% CI: 14.2%, 20.6%), 33.2% (95% CI: 29.6%, 37.1%), and 22.7% (95% CI: 19.5%, 26.2%), respectively. The adjusted geometric means of the RBC folate concentration increased significantly (P -trend < 0.001) in WCBA who had normal vitamin B-12 status relative to WCBA who were vitamin B-12 deficient.

Conclusions—In Belize, the prevalence of folate and vitamin B-12 deficiencies continues to be a public health concern among WCBA. Furthermore, low folate status co-occurred with low vitamin B-12 status, underlining the importance of providing adequate vitamin B-12 and folic acid intake through approaches such as mandatory food fortification.

Keywords

anemia; folate deficiency; folate insufficiency; vitamin B-12 deficiency; women of childbearing age

Introduction

Folate deficiency, vitamin B-12 deficiency, and anemia can have adverse effects on birth outcomes (1, 2). In particular, neural tube defects (NTDs)¹¹ are associated with both inadequate folate and vitamin B-12 status of the mother (3–7) and the infant (8–10). Inadequate folate status can slow DNA synthesis and cell growth (through the one-carbon metabolism pathway), as well as the formation and maturation of RBCs. In addition, because vitamin B-12 is necessary for methionine synthase-based activation of folate and subsequent entry into one-carbon metabolism, a low concentration of vitamin B-12 can contribute to the reduced formation of metabolically active folate, reduced folate retention in developing RBCs, and indirect induction of intracellular folate deficiency. Low vitamin B-12 status is also an independent risk factor for an NTD-affected pregnancy (5, 10–12). However, the interaction between folate and vitamin B-12 concentrations has not been examined at a population level and may be important for predicting NTD risk and evaluating prevention efforts.

Evidence suggests that NTD risk can be reduced if women consume recommended amounts of folic acid periconceptionally (5, 13, 14). To prevent folate-sensitive NTDs, the US Public Health Service recommended in 1992 that all women capable of becoming pregnant consume 400 µg folic acid daily (13). Furthermore, the WHO recently recommended an RBC folate concentration of 906 nmol/L (400 ng/L) as the threshold for the optimal concentration for NTD risk reduction (15). Many countries have enacted legislative

¹¹Abbreviations used: BLZ\$, Belize dollars; MBA, microbiologic assay; NTD, neural tube defect; PPS, probability proportional to size; PRR, prevalence risk ratio; RPBA, radioproteinbinding assay; WCBA, women of childbearing age.

regulations and/or micronutrient fortification programs that include folic acid to improve the folate status of women of childbearing age (WCBA) (16). However, few fortification programs have established monitoring and evaluation to assess the impact of increased folic acid intake on the folate status within their populations. The potential impact of folic acid fortification programs on NTD prevalence is also largely unknown in most low-to-middle income countries because of limited birth-defect surveillance programs (17). Furthermore, few countries have included vitamin B-12 in their fortification efforts.

In 2007 Belize established a voluntary wheat flour fortification program as a result of the Central American fortification harmonization strategy adopted by the Ministros de Salud de Centro America in 2002 (18). The Central American harmonization strategy recommended that wheat flour be fortified with the following nutrients (and amounts): thiamin (6.2 mg/kg), riboflavin (47.2 mg/kg), niacin (55.0 mg/kg), folic acid (1.8 mg/kg), and iron (55.0 mg/kg). Belize's voluntary wheat-flour fortification program does not have a monitoring and evaluation component to ensure proper fortification concentrations or access to fortified wheat flour for the population.

Before establishing a mandatory program, the government wished to establish the baseline micronutrient status among nonpregnant WCBA. To address this, the Belizean government conducted the Belize National Micronutrient Survey. The objectives of this study were to establish the baseline folate status, the vitamin B-12 status, and the prevalence of anemia among WCBA and examine the association between folate and vitamin B-12 status by sociodemographic characteristics and geographic regions.

Methods

The Belize Institutional Review Board reviewed and approved the study protocol in January 2011. The study protocol also was reviewed and approved by the CDC Institutional Review Board in Atlanta (Georgia). In addition, written consent was obtained from the participating women.

Belizean population

In 2010 Belize had a total population of 310,896. Women made up 49.4% of the total population, and WCBA made up 27.3% of the total population. At that time, Belize consisted of the following diverse ethnic groups: Mestizos (52%), Mayans (11%), Creoles and Garifunas (27%), and Mennonites, East Indians, Chinese, and Europeans (10%). An estimated 44.5% of the population lived in urban settings, and 55.5% in rural areas (19).

Sampling design

A cross-sectional population survey was conducted among noninstitutionalized WCBA (aged 15–49 y) during the period March–June 2011. Because of regional differences in the distribution of rural compared with urban populations and indigenous populations in the country, a stratified, multistage probability sample was used for each of the 4 regions: Central, North, South, and West. Two hundred twenty sectors, 55 in each region, were selected by using probability-proportional-to-size (PPS) sampling (20, 21). The first stage of the 3-stage sample design was a selection of primary selection units with PPS sampling

stratified by urban or rural settings. In the second stage, block groups were selected for each sector by using PPS sampling of each secondary sampling unit within each selected primary selection unit. All households within the selected block group were listed and updated. In the third stage of sampling, households within each selected block group were sampled by using a random start point and pre-established systematic selection of adjacent homes. Households were visited by trained interviewers to determine their eligibility, defined as having 1 woman 15–49 y of age residing in the household. The minimum sample size per region to estimate a 50% prevalence of folate insufficiency or vitamin B-12 deficiency and marginal deficiency with a standard error of 7.5%, given a survey design effect of 1.4, was 266 individuals in each region after adjusting for nonresponse. The final sample provided sufficient power to obtain reliable national and regional estimates.

Household interviews

For the households selected in each of the block groups, interviewers used a standardized protocol, proceeded door-to-door to explain the study to each household, and identified all eligible women residing in the household. When 2 eligible women were identified in a selected household, one woman was chosen through a random selection process. If a selected woman was not available at initial contact, 2 more attempts were made at various hours and days. If the woman was never contacted, the household was classified as a nonresponse household.

With the use of a standardized questionnaire, interviewers collected sociodemographic information, the number of women and children who were household members, primary household language, occupation and educational level of the index woman, total family income, use of health services, and vitamin use. Pregnancy status was determined by self-report. Household interviews were conducted in English, Spanish, or Mayan language by multilingual interviewers.

Variable definitions

Self-reported data for the following sociodemographic information were obtained from the women interviewed: 1) Area was predetermined and defined by the location of the household. Urban areas included towns, small cities, and large cities with populations ≥ 2500 . Rural areas were locations with populations <2500 . 2) Ethnicity was self-identified (Creole, Garifuna, Mayan, Mestizo, White, and Asian, or Other). Those who self-identified as Creole or Garifuna were categorized as Creole/Garifuna, and those who identified as White, Asian, or Other were categorized as Other. 3) Age was grouped into 1 of 3 categories: 15–24, 25–34, and 35–49 y. 4) Education level was based on the highest grade or level of education the woman had attained. 5) Monthly family income was grouped into one of 4 categories of Belize dollars (BLZ\$): ≤ 699 , 700–1399, or >1399 BLZ\$, and Don't Know (the respondent did not know the family income). 6) Use of health services was categorized as Private, Private and Public, Public, and Other. 7) Intake of a folic acid-containing supplement during the last year was categorized as either Yes or No.

After the short interview was completed, blood samples were drawn for measurement and assessment of nutritional biomarkers.

Blood collection and processing

Because of the nature of the survey, blood samples were taken under non-fasting conditions. Blood samples were taken by venipuncture into tubes containing EDTA. Whole-blood vacutainers were maintained at 4°–8°C for up to several hours before transport to field laboratories. At the field laboratories, hemoglobin determination was performed by using HemoCue, serum samples were separated and aliquoted, and whole-blood EDTA hemolysates were prepared in accordance with CDC laboratory specifications (22). Samples were subsequently transported at –20°C to the central Belize Ministry of Health where they were stored at –80°C until shipped on dry ice to the University of Florida laboratory in Gainesville (Florida).

Biochemical analyses

During the period 2011–2013, the University of Florida laboratory conducted biochemical analyses for plasma vitamin B-12 by using the Roche electrochemiluminescence assay on the E-170 instrument platform (Roche Vitamin B-12 assay package insert, 2007–8, V4). Serum and RBC folate concentrations were determined by microbiologic assay (MBA) (*Lactobacillus rhamnosus* 27773; ATCC) by using the microtiter plate adaptation of O’Broin et al. (23). The intra- and inter assay CVs for the serum folate quality-control standards (24) were 4.7%, 5.8%, and 7.5% and 7.2%, 10.8%, and 10.6% for the high, medium, and low standards, respectively. The intra- and interassay CVs for the RBC folate-positive controls were 5.5%, 6.1%, and 5.1% and 8.0%, 9.1%, and 8.7% for the high, medium, and low standards, respectively.

Biochemical indicators

For the purposes of this survey, the prevalence of folate deficiency was assessed by using both the WHO–radioproteinbinding assay (RPBA) cutoffs of <10 nmol/L for serum folate and <340 nmol/L for RBC folate (25) and the assay-matched cutoffs for the MBA of 14 nmol/L for serum folate and <624 nmol/L for RBC folate from Pfeiffer et al. (26). In addition, RBC folate insufficiency was defined based on the recent WHO guideline establishing a threshold for the optimal RBC folate concentration for NTD risk reductions (<906 nmol/L) (15). The folate insufficiency cutoff (906 nmol/L) was adjusted for differences between the Irish MBA and the MBA as reported previously, resulting in an assay-adjusted cutoff for folate insufficiency of <748 nmol/L (27). The cutoff for identifying vitamin B-12 deficiency was <148 pmol/L, and it was 148–221 pmol/L for marginal vitamin B-12 deficiency (28). Anemia was defined by using WHO standards for hemoglobin concentrations in nonpregnant women (<12 g/dL) (29). Hemoglobin concentrations were adjusted for altitude as recommended by the WHO (29). A 5% threshold criterion has been established, above which prevalence estimates for folate deficiency (serum and RBC folate) and insufficiency (RBC folate), vitamin B-12 deficiency, and anemia were indicative of a public health concern (30).

Statistical methods

Statistical analyses were performed with SAS 9.3 (SAS Institute) and SUDAAN (version 11.0; Research Triangle Institute). Sample weights and sample design variables were

used to produce nationally and regionally representative estimates that account for the clustered design and adjustments for household nonresponse (21). Descriptive analyses included national frequencies, percentages, and 95% CIs for sociodemographic variables (area, ethnicity, age, education, family income, reported use of folic acid-containing supplements, and use of health services) and region. The crude prevalence estimates and 95% CIs for folate deficiency (serum and RBC concentrations), RBC folate insufficiency, vitamin B-12 deficiency and marginal deficiency, and anemia were determined. Comparisons with the selected variables were done by using prevalence risk ratios (PRRs) for serum and RBC folate and vitamin B-12 deficiencies. Adjusted PRRs were calculated by using logistic regression models to estimate the probability of RBC folate insufficiency and vitamin B-12 status (deficiency and marginally deficient). Adjusted PRRs were estimated from the logistic regression models by using the PREDMARG statement in SUDAAN (31). The adjusted PRRs were estimated by using the logistic regression model to estimate the probability of deficiency status, taking into account the covariates and the survey design.

Geometric means were used because distributions of blood folate and vitamin B-12 concentrations were skewed. Geometric means by sociodemographic and other characteristics by using least mean square error algorithms derived from linear regression models (Proc Regress SUDAAN) were calculated as well. The 95% CIs for geometric means were constructed with Wald's method (32). The Taylor series approximation was used to obtain the SEs (33). Pairwise differences were tested for geometric means of biomarker concentrations within each sociodemographic characteristic by using *t* tests derived from linear regressions to adjust for the selected variables.

Selected percentiles were estimated for overall serum and RBC folate and vitamin B-12 concentrations, as well as the percentiles stratified by selected sociodemographic characteristics. RBC folate concentrations were assessed while stratifying by vitamin B-12 status (deficient, marginally deficient, and normal) and by selected sociodemographic characteristics. In addition, linear trends for RBC folate concentrations by vitamin B-12 status (treated as an ordinal variable) were tested by using multiple linear regression and adjusted by sociodemographic characteristics and region with the Satterwaite adjusted *F*-statistic (34). All reported *P* values were based on 2-sided tests.

Results

Response rates

A total of 1869 households were identified. All households were contacted to determine their eligibility to participate in the study. In the end, 1156 households (61.9%) had one eligible WCBA. Of these, 88.6% agreed to participate. This yielded a total of 937 WCBA.

Characteristics of the study population

Table 1 summarizes the characteristics of the sample population: 51.3% lived in rural areas, 53.2% were identified as of Mestizo ethnicity, 37.7% were 15–24 y of age, 23.1% had <6 y of education, and 29.9% reported a monthly family income of 699 BLZ\$. In addition, 67.0% reported the use of public health services only, 43.6% reported intake of folic acid–

containing supplements, and 32.6% resided in the Central region. The Belize sample population reflected the Belizean general population of WCBA at the time (Supplemental Table 1). However, our sample population was less educated than the overall female Belizean population. Also, the ethnic distribution varied by region: The Central region was predominantly Black (Creole/Garifuna) and Mestizo (47.0% and 43.4%, respectively), the North and West regions were predominately Mestizo (80.7% and 61.4%, respectively), and the South region was predominantly Mayan (49.0%).

Prevalence of deficiencies and geometric means for serum and RBC folate, vitamin B-12, and anemia

The national prevalence estimates for serum folate deficiency and RBC folate deficiency by using the WHO-recommended cutoffs for deficiency were 4.1% (95% CI: 2.7%, 6.3%) and 6.8% (95% CI: 4.9%, 9.4%), respectively, and by using the cutoffs from Pfeiffer et al. (26) were 11.0% (95% CI: 8.6%, 14.0%) and 35.1% (95% CI: 31.3%, 39.2%), respectively (Table 2). In addition, the prevalence for RBC folate insufficiency was 48.9% (95% CI: 44.8%, 53.1%). The prevalence estimates for vitamin B-12 deficiency and marginal deficiency were 17.2% (95% CI: 14.2%, 20.6%) and 33.2% (95% CI: 29.6%, 37.1%), respectively. The prevalence of anemia was 22.7% (95% CI: 19.5%, 26.2%). The adjusted geometric means were 28 nmol/L (~95% CI: 26, 29 nmol/L) for serum folate, 719 nmol/L (~95% CI: 689, 750 nmol/L) for RBC folate, 233 pmol/L (~95% CI: 223, 244 pmol/L) for plasma vitamin B-12, and 12.7 g/dL (~95% CI: 12.5, 12.8 g/dL) for hemoglobin (Table 2). The percentile distributions for serum folate, RBC folate, and vitamin B-12 are given in Supplemental Table 2.

RBC folate insufficiency and vitamin B-12 deficiency and marginal deficiency by area, ethnicity, age, education level, income, and region

The prevalence estimates and PRRs for RBC folate insufficiency ranged from 33.5% to 68.7% (Table 3) among the different subgroups. There was little variation by population characteristic except for a significantly higher insufficiency among Mayan WCBA than other ethnic groups [PRR 1.9 (95% CI: 1.2, 3.0)]. Plasma vitamin B-12 deficiency and marginal deficiency showed variations across some population characteristics and regions (Table 4). For vitamin B-12 deficiency, the only adjusted PRRs reaching statistical significance were for WCBA of Mayan and Mestizo origin [3.3 (95% CI: 1.3, 8.5) and 2.4 (95% CI: 1.1, 5.3), respectively], WCBA with 7–12 y of education [2.2 (95% CI: 1.05, 4.7)], and WCBA in the South region [0.5 (95% CI: 0.3, 0.9)]. For marginal vitamin B-12 deficiency, the only adjusted PRRs reaching significance were for WCBA of Mayan and Mestizo origin [2.1 (95% CI: 1.3, 3.5) and 1.9 (95% CI: 1.2, 2.9)] and for WCBA in the South region, with a significantly lower prevalence [0.7 (95% CI: 0.5, 0.8)].

Distribution of geometric means of serum and RBC folate and plasma vitamin B-12 concentrations by sociodemographic characteristics

Variations in adjusted geometric mean concentrations and percentile distributions for serum and RBC folate and plasma vitamin B-12 were noted across some population characteristics and regions. Geometric mean concentrations of RBC folate were significantly lower among WCBA who were of Mayan origin and young and were significantly lower among WCBA in

the South and West regions (Table 5). Geometric mean concentrations of plasma vitamin B-12 were significantly lower among WCBA who were of Mayan origin and in the North region and were significantly higher among WCBA who used private health services and who reported taking a folic acid-containing supplement. Geometric mean concentrations of serum folate were significantly lower among WCBA aged 25–34 y, among WCBA with <6 y of education, among WCBA with family incomes of 700–1399 BLZ\$, and among WCBA residing in the Central, South, and West regions (Supplemental Table 3).

Distribution of geometric means of RBC folate concentrations stratified by vitamin B-12 status and selected population and geographic characteristics

Because of the high prevalence of vitamin B-12 deficiency and marginal deficiency among this population, adjusted geometric means of RBC folate concentration of participants were stratified according to vitamin B-12 status (i.e., deficient, marginally deficient, normal) and other participant characteristics (Figure 1, Supplemental Table 4). Overall, the national-adjusted geometric means of RBC folate concentration increased significantly ($P_{\text{trend}} < 0.001$) from 610 nmol/L among WCBA who were vitamin B-12 deficient to 641 nmol/L among WCBA with marginal vitamin B-12 deficiency to 783 nmol/L among WCBA who had a normal vitamin B-12 status. The trend was similar within most sociodemographic subgroups, with RBC folate concentration increasing across the 3 categories of vitamin B-12 status. However, a statistically significant trend was not observed among the Mayan, Creole/Garifuna, and Other ethnicity categories; among those 15–24 y old; those with 12 y education; those with family incomes of 700 BLZ\$; among those using public/private or other health services; and among those residing in the Central and South regions.

RBC folate concentrations for each vitamin B-12 status and sociodemographic subgroup also were compared with the folate-insufficiency threshold (Figure 1). Adjusted geometric means of RBC folate concentrations were above the threshold for insufficiency (thus within the optimal range) for WCBA with normal vitamin B-12 levels nationally, for most demographic groups, and in the North region. In contrast, although RBC folate concentrations of WCBA of Mayan origin, WCBA who were users of private and private/public health services, and WCBA residing in the South and West regions increased with improved vitamin B-12 status, they remained below the threshold for folate sufficiency for almost all vitamin B-12 subgroups.

Discussion

To our knowledge, this is Belize's first national and regional premandatory fortification baseline survey to present data on folate status, vitamin B-12 status, and anemia prevalence among nonpregnant WCBA. It is also the first population-based survey, to our knowledge, to examine the association of vitamin B-12 status with RBC folate concentrations among nonpregnant WCBA. The results indicate that despite voluntary fortification of wheat flour, the prevalence estimates for folate and vitamin B-12 deficiency are much higher than the WHO threshold for public health concern. In particular, the greater prevalence of both folate and vitamin B-12 deficiency may lead to a higher incidence of NTDs and other adverse birth

outcomes in this population. In addition, anemia remains a moderate public health concern (29).

Folate and vitamin B-12 concentrations

National serum and RBC folate and vitamin B-12 concentrations among nonpregnant WCBA in our study were similar to or slightly lower than those reported from the postfortification period in Guatemala (28 compared with 30 nmol/L, 719 compared with 725 nmol/L, and 233 compared with 341 pmol/L, respectively) (35). Also, we observed lower RBC folate and/or plasma vitamin B-12 concentrations in urban areas, among WCBA of Mayan origin, and in the West and South regions. These results were consistent with published results from Guatemala, other Latin American countries, and the United States (35–40), where variability of blood folate and vitamin B-12 concentrations within the population, as well as across racial and ethnic groups and regions, was observed. These differences could be explained in part by cultural differences and/or differences in access to or availability of fortified wheat flour for vulnerable populations (35).

Prevalence of folate deficiency and insufficiency, vitamin B-12 deficiency and marginal deficiency, and anemia

Comparison of folate status based on serum and RBC folate concentrations requires the use of the same assay and cutoffs to compare results within and between countries, it should discriminate between at-risk groups and those not at risk, and it should be validated through clinical trials (i.e., controlled folate depletion studies in which changes in clinical indexes are closely aligned with requirement). In our study, 2 different sets of cutoffs, those published by the WHO (25) and the assay-matched cutoffs of Pfeiffer et al. (26), were used to determine folate status. The WHO cutoffs are based on cross-sectional data from NHANES 1988–1994 that associated rising plasma homocysteine as a metabolic indicator of insufficient serum and RBC folate concentrations measured by using a commercial RPBA. This commercial assay grossly underestimates blood folate concentrations in a variable manner depending on genotype for a very common folate polymorphism (5,10-methylenetetrahydrofolate reductase C677T), and the product was discontinued a decade ago. In contrast to the WHO cutoffs, the Institute of Medicine (41) previously reported cutoffs from controlled folate-depletion feeding studies in which folate was measured by MBA and cutoffs were defined based on hematological changes indicative of deficient erythropoiesis with the final stage being megaloblastic anemia. In our study, a substantially higher prevalence of folate deficiency based on serum (6.9% absolute difference) and RBC folate (28.9% absolute difference) concentrations was observed by using the assay-matched cutoffs from Pfeiffer et al. (26) compared with the WHO cutoffs (25). Thus, the prevalence of folate deficiency in Belize appears to be substantially higher than what the WHO cutoffs would suggest. This underscores the inappropriateness of using cutoffs based on the commercial RPBA to define folate status in studies in which blood folate was measured by using an MBA.

To compare like to like, our study compared results that used the WHO recommended cutoffs (25) to the results of other studies that used the same cutoffs. Belize, a country with a voluntary fortification program, had higher prevalence of serum folate deficiency (4.1%)

than several countries that have mandatory fortification, including the Dominican Republic (3.1%) (38), Mexico (1.9%) (42), Nicaragua (2.3%) (37), and the United States (<1%) (39). Similarly, the prevalence of RBC folate deficiency was greater in Belize (6.8%) than in Nicaragua (1.1%) (37) and the United States (<1%) (39). The amount of folic acid fortification in the aforementioned countries with mandatory fortification programs ranges between 140 and 180 mg/kg, and most of these countries fortify wheat and corn flour at a minimum. Surprisingly, folate deficiency was higher in 2 countries than in Belize (Guatemala: 5.1% or 8.9%, based on serum or RBC folate concentrations, respectively, and the Dominican Republic: 7.0% based on RBC folate concentrations) (35, 38). Differences between countries could be explained by differences in country characteristics and differences in their populations' access to fortified staples.

By using the WHO threshold (15) as an indicator of optimal folate status, prevalence of folate insufficiency was observed among 49% of WCBA in Belize, placing them at a higher risk of NTD-affected pregnancy. This is an important public health concern (30). The prevalence of RBC folate insufficiency was slightly higher than that reported by Guatemala (47.2%) (43) but was much higher than that reported by the United States (22.1%) (27). Thus, with Belize's voluntary folic acid fortification program, there is a high prevalence of folate deficiency and insufficiency. Because of this, many women are at greater risk of having an NTD-affected pregnancy. Guatemala's program, although mandatory, has also had difficulty in reducing folate insufficiency. In both countries, vulnerable populations, such as the Mayans or the poor, have inadequate access to fortified wheat flour, limited purchasing power, and/or do not consume wheat flour as their main food staple (44). Fortification of additional staples, such as corn flour or rice, may be needed to reach at-risk populations and reduce folate insufficiency and prevent folate-sensitive NTDs. In contrast, the US population is more homogenous in its dietary practices and access to fortified staples. Also, the US population consumes multiple fortified staples, including breakfast cereals, which likely explains its lower prevalence of folate insufficiency (45).

Our study also found that the prevalence of vitamin B-12 deficiency and marginally deficient status (17.2%, and 33.2%, respectively) were of public health concern, although similar to those reported in Guatemala and other Latin American countries (35, 36, 40). Furthermore, vitamin B-12 concentrations in our study were lower than those reported from Guatemala (233 compared with 341 pmol/L). These differences are likely to be explained by a lower intake of meat products (40), suggesting that other sources of vitamin B-12, such as fortified food, may be necessary to address vitamin B-12 deficiency.

Anemia is also a public health concern in Belize with a prevalence of 22.7% among WCBA. The prevalence of anemia in Belize was higher than that reported in the United States (12%) (46), Guatemala (21.4%) (47), El Salvador (12%) (48), Costa Rica (10.2%) (48), and Nicaragua (11.2%) (48) and lower than that reported in the Dominican Republic (34%) (38) and Mexico (29.4%) (42). Of note, an elevated prevalence of anemia has persisted in these regions for many years despite efforts to increase iron intake (49).

RBC folate concentrations stratified by vitamin B-12 status

In this study, WCBA characterized as vitamin B-12 deficient or marginally deficient did not have optimal RBC folate concentrations for reducing the risk of NTD-affected pregnancies. This finding was consistent across most sociodemographic subgroups (Figure 1) and is consistent with what is known about the interaction between vitamin B-12 and folate in the one-carbon metabolism pathway. Vitamin B-12 deficiency interferes with the one-carbon cycle by reducing the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate and trapping folate in a metabolically less active form. As a result, DNA synthesis is reduced, thereby limiting the number of cells that can divide. Molloy et al. (5) reported that pregnant women with low vitamin B-12 concentrations [<250 ng/L (<184.5 pmol/L)] also had lower RBC folate concentrations and had a 2.5-times higher risk of giving birth to a child with an NTD.

Strengths and limitations

Our study has several major strengths. To our knowledge, it is the first study in Belize to assess micronutrient status at the national and regional levels among WCBA. This is especially important as Belize will be implementing mandatory fortification in the near future, and these results will provide a baseline against which future (postmandatory fortification) survey results can be compared. This study was designed and implemented by using a valid and reliable population-based sampling design and strict field and laboratory methodologies with the intent of reducing biases in the selection of households and individuals and to ensure proper handling of biological samples. Moreover, folate analysis was performed by using the MBA, which is considered the gold standard for determining circulating folate concentrations, particularly RBC folate because it measures all forms of folate exhibiting biological activity (50), thereby reducing biases because of the effect of the 5,10-methylenetetrahydrofolate reductase gene polymorphism (15).

The study also has limitations, such as the lack of additional biomarkers of vitamin B-12 (e.g., methylmalonic acid or holotranscobalamin) for improved status assessment, and the lack of data regarding dietary intake, an important component of nutrient status assessment. Somewhat surprisingly, self-reported use of folic acid-containing supplements was not correlated with measurements of blood folate. This suggests the limited reliability of using a self-reported measurement of supplement use without confirmatory evidence. In addition, the study included a relatively smaller sample size than required for some regions, which limited the precision for the estimation of biomarkers, resulting in wider CIs (the Central and, to a lesser degree, the North regions).

Conclusions

In Belize, the prevalence of folate and vitamin B-12 deficiencies continues to be a public health concern for WCBA. Folate deficiency and insufficiency were more prevalent among the Mayan population and women who were residing in the South region. Vitamin B-12 deficiency was also widespread among WCBA. This study illustrates that when the assay type is matched with the appropriate assay cutoff, the prevalence of folate deficiency or insufficiency is higher than what would be reported by using mismatched cutoffs. In addition, this study provides evidence of the potential co-occurrence of low vitamin B-12

status and folate status, thus underlining the importance of providing adequate amounts of vitamin B-12 and folic acid to support NTD risk reduction. To assist in accomplishing this, Belize may benefit from mandatory micronutrient fortification programs that include the addition of folic acid and vitamin B-12 to staple foods such as rice and corn in addition to wheat flour to achieve sufficient intake of these nutrients among the population at risk. Finally, this study provides baseline information that will be useful for monitoring the effectiveness of future folic acid and vitamin B-12 fortification intervention programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The authors' responsibilities were as follows—JR, NL, LBB, GPAK, DO, and PA: planned and designed the study; JR, NL, and DO: managed data collection and data entry; LBB and GPAK: processed folate and vitamin B-12 blood samples; JR, CJA, and MC: carried out data analysis; JR, MC, LBB, GPAK: carried out data interpretation and drafted the manuscript; JS, RF, RD, and PA: assisted in data interpretation and manuscript drafting; and all authors: read and approved the final manuscript as submitted.

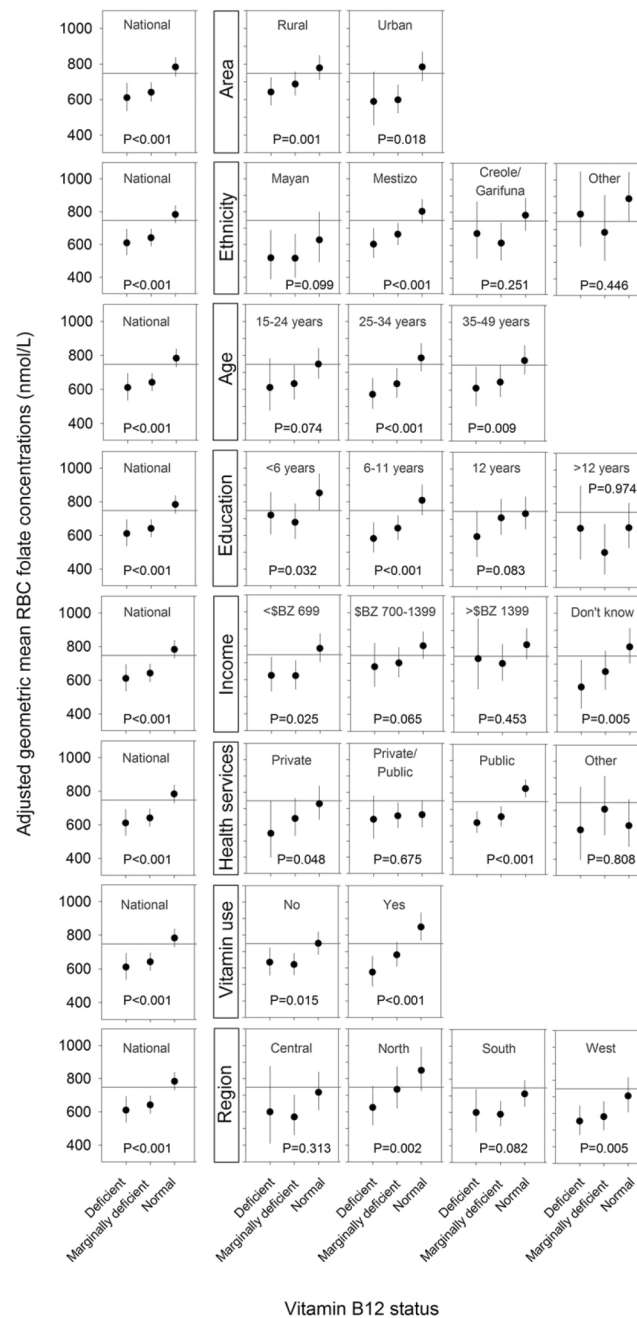
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**FIGURE 1.**

Adjusted geometric means and 95% CIs of RBC folate concentrations by vitamin B-12 status and sociodemographic characteristics and region in women of childbearing age in Belize. The RBC folate optimal threshold is shown with a horizontal line. \$BZ, Belize dollars.

TABLE 1

Demographic and other characteristics of nonpregnant women of childbearing age, Belize 2011

Characteristic	Unweighted n (N = 937)	Weighted % (95% CI)
Area		
Rural	559	51.3 (43.8, 58.6)
Urban	378	48.7 (41.4, 56.2)
Ethnicity		
Mayan	139	12.3 (9.3, 16.1)
Mestizo	468	53.2 (47.7, 58.6)
Black (Creole/Garifuna)	258	26.3 (21.8, 31.3)
Other	72	8.2 (5.7, 11.7)
Age, y		
15–24	294	37.7 (33.7, 41.9)
25–34	343	29.8 (26.5, 33.1)
35–49	300	32.5 (28.7, 36.5)
Education, y		
<6	233	23.1 (19.6, 27.1)
6–11	354	37.9 (33.4, 42.7)
12	223	25.8 (22.0, 30.0)
>12	127	13.2 (10.2, 16.8)
Monthly family income (BLZ\$) ^I		
Don't know	207	23.2 (19.5, 27.3)
699	290	29.9 (25.9, 34.1)
700–1399	248	26.5 (22.9, 30.4)
>1399	191	20.4 (17.1, 24.2)
Health services use		
Private	125	13.2 (10.8, 16.0)
Private/public	100	10.3 (7.9, 13.4)
Public	635	67.0 (62.3, 71.4)
Other	77	9.4 (6.9, 12.9)
Use of folic acid-containing supplements in the last year		
No	538	56.4 (52.0, 50.7)
Yes	399	43.6 (39.2, 48.0)
Region		
Central	223	32.6 (28.7, 36.7)
North	208	26.3 (23.3, 29.6)
South	243	17.7 (15.4, 20.3)
West	263	23.4 (20.8, 26.1)

^I 1 US\$ = 2 BLZ\$.

One missing value.

BLZ\$, Belize dollar.

TABLE 2

Geometric mean concentrations of folate, vitamin B-12, and anemia and prevalence of deficiency among nonpregnant women of childbearing age, Belize 2011

Indicator	Unweighted <i>n</i>	Geometric mean (95% CI)	Prevalence (95% CI)
Serum folate	937	28 (26, 30)	
Deficiency (<10 nmol/L) ¹	46	8 (8, 9)	4.1 (2.7, 6.3)
Deficiency (<14 nmol/L) ²	117	10 (10, 11)	11.0 (8.6, 14.0)
RBC folate	937	719 (689, 750)	
Deficiency (<340 nmol/L) ¹	61	265 (252, 278)	6.8 (4.9, 9.4)
Deficiency (<624 nmol/L) ²	320	441 (422, 461)	35.1 (31.3, 39.2)
Insufficiency (<906 nmol/L) ³	632 ⁴	573 (552, 594)	68.5 (64.6, 72.2)
Insufficiency (<748 nmol/L) ²	461 ⁴	503 (483, 523)	48.9 (44.8, 53.1)
Plasma vitamin B-12	930 ⁵	233 (223, 244)	
Deficient (<148 pmol/L) ⁶	160	121 (117, 125)	17.2 (14.2, 20.6)
Marginally deficient (148–221 pmol/L) ⁶	294	185 (183, 187)	33.2 (29.6, 37.1)
Hemoglobin	931 ⁵	12.7 (12.5, 12.8)	
Anemia (<12 g/dL) ⁷	197	10.8 (10.6, 11.0)	22.7 (19.5, 26.2)

¹WHO (25).

²Pfeiffer (26).

³WHO (15).

⁴Includes those with RBC folate deficiency.

⁵Blood samples available.

⁶Allen (28).

⁷WHO (29).

TABLE 3Prevalence and aPRRs of RBC folate insufficiency in nonpregnant women of childbearing age, Belize 2011[/]

Characteristic	RBC folate insufficiency (<748 nmol/L)		
	Unweighted <i>n</i>	Prevalence (95% CI)	aPRR (95% CI) ²
National	937	48.9 (44.8, 53.1)	
Area			
Rural	559	49.1 (43.9, 56.1)	0.9 (0.8, 1.1)
Urban	378	50.6 (44.5, 56.7)	Referent
Ethnicity			
Mayan	139	70.5 (60.1, 79.2)	2.4 (1.4, 3.4) ³
Mestizo	468	46.2 (40.2, 52.3)	1.4 (0.9, 2.2)
Black (Creole/Garifuna)	258	54.3 (46.3, 62.1)	1.6 (1.0, 2.5)
Other	72	33.5 (21.0, 48.8)	Referent
Age, y			
15–24	294	55.5 (48.2, 62.6)	1.2 (1.0, 1.4)
25–34	343	48.7 (42.0, 55.3)	1.0 (0.9, 1.3)
35–49	300	45.7 (38.6, 52.9)	Referent
Education, y			
<6	233	47.4 (39.4, 55.6)	1.0 (0.7, 1.3)
6–11	354	53.7 (46.7, 60.5)	1.1 (0.8, 1.5)
12	223	50.8 (42.6, 58.9)	1.0 (0.8, 1.4)
>12	127	44.4 (33.5, 55.9)	Referent
Monthly family income, BLZ\$			
Don't know	207	48.5 (40.5, 56.6)	1.0 (0.8, 1.3)
699	290	51.9 (44.0, 59.6)	1.0 (0.8, 1.3)
700–1399	248	53.4 (45.0, 61.0)	1.1 (0.8, 1.4)
>1399	191	45.9 (36.8, 55.3)	Referent
Health services use			
Private	125	42.9 (33.2, 53.5)	0.9 (0.7, 1.2)
Private/public	100	59.5 (47.9, 70.2)	1.3 (1.1, 1.6) ³
Public	635	50.7 (45.6, 55.4)	Referent
Other	77	47.3 (32.3, 62.8)	0.9 (0.6, 1.3)
Use of folic acid-containing supplements in the last year			
No	538	53.0 (46.7, 59.2)	1.1 (0.9, 1.3)
Yes	399	48.1 (46.3, 57.3)	Referent
Region			
Central	208	50.3 (41.6, 59.0)	1.2 (0.9, 1.5)
North	223	40.2 (32.3, 48.7)	Referent
South	243	60.4 (51.5, 68.7)	1.2 (0.9, 1.6)
West	263	53.9 (46.8, 60.8)	1.2 (0.9, 1.6)

[/] aPRR, adjusted prevalence risk ratio; BLZ\$, Belize dollar.

²Adjusted by area, ethnicity, age, education, income, and region.

³ $P < 0.0001$.

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Prevalence and aPRRs for vitamin B-12 deficiency in nonpregnant women of childbearing age, Belize 2011¹

TABLE 4

Characteristic	<i>n</i> (unweighted)	Vitamin B-12 deficient (<148 nmol/L)		Vitamin B-12 marginally deficient (148–221 pmol/L)	
		Prevalence	aPRR (95% CI) ²	Prevalence	aPRR (95% CI)
National Area	937	17.2 (14.2, 20.6)		33.2 (29.6, 37.1)	
Rural	559	19.3 (15.2, 24.2)	1.2 (0.9, 1.8)	32.2 (27.8, 37.1)	0.9 (0.7, 1.1)
Urban	378	14.9 (10.9, 24.2)	Referent	34.3 (28.6, 40.5)	Referent
Ethnicity					
Mayan	139	19.7 (12.2, 30.4)	3.3 (1.3, 8.5) ***	33.7 (25.7, 42.7)	2.1 (1.3, 3.5) **
Mestizo	468	19.4 (15.3, 24.3)	2.4 (1.1, 5.3) **	39.0 (33.3, 45.2)	1.9 (1.2, 2.9) **
Black (Creole/Garifuna)	258	14.3 (9.2, 21.5)	2.2 (0.9, 5.6)	23.9 (18.5, 30.3)	1.2 (0.7, 1.3)
Other	72	8.2 (3.8, 16.5)	Referent	24.7 (15.8, 36.3)	Referent
Age, y					
15–24	294	15.8 (10.4, 23.3)	0.8 (0.5, 1.3)	33.8 (27.2, 41.1)	0.9 (0.7, 1.3)
25–34	343	17.1 (13.1, 22.1)	0.9 (0.7, 1.3)	32.9 (27.2, 39.2)	1.0 (0.7, 1.3)
35–49	300	18.8 (14.3, 24.2)	Referent	32.8 (25.9, 40.6)	Referent
Education, y					
<6	233	20.0 (14.5, 26.9)	1.9 (0.9, 4.3)	33.1 (26.6, 40.4)	0.9 (0.6, 1.3)
6–11	354	17.1 (12.8, 22.4)	1.8 (0.9, 3.8)	30.8 (25.1, 37.2)	0.9 (0.6, 1.2)
12	223	19.2 (13.2, 27.2)	2.2 (1.05, 4.7) **	34.2 (26.2, 43.4)	1.0 (0.7, 1.5)
>12	127	8.5 (4.5, 15.4)	Referent	38.5 (28.5, 49.6)	Referent
Monthly family income, BLZ\$					
Don't know	207	21.3 (14.6, 30.1)	1.2 (0.7, 2.2)	29.1 (22.3, 37.0)	0.9 (0.7, 1.4)
699	290	17.3 (12.5, 23.5)	0.9 (0.6, 1.6)	33.0 (26.1, 40.8)	1.0 (0.7, 1.4)
700–1399	248	15.5 (11.0, 21.3)	0.8 (0.5, 1.5)	35.4 (28.2, 43.4)	1.0 (0.8, 1.4)
>1399	191	14.5 (9.2, 22.1)	Referent	35.4 (26.9, 44.9)	Referent
Health services use					
Private	125	15.2 (8.9, 24.6)	0.7 (0.4, 1.0)	25.4 (16.8, 36.3)	0.7 (0.5, 1.1)
Private/public	100	7.8 (3.9, 15.0)	1.1 (0.8, 1.4)	42.4 (31.9, 53.6)	1.3 (0.8, 1.5)
Public	635	18.7 (15.1, 22.8)	Referent	32.2 (27.9, 36.9)	Referent

Characteristic	n (unweighted)	Vitamin B-12 deficient (<148 nmol/L)		Vitamin B-12 marginally deficient (148–221 pmol/L)	
		Prevalence	aPRR (95% CI) ²	Prevalence	aPRR (95% CI)
Other	77	19.6 (9.6, 35.9)	1.2 (0.9, 1.7)	41.1 (28.9, 54.4)	1.2 (0.9, 1.7)
Use of folic acid-containing supplements in the last year					
No	536	19.2 (14.9, 24.4)	1.3 (0.9, 1.9)	35.2 (30.4, 40.3)	1.1 (0.9, 1.5)
Yes	394	12.2 (9.0, 16.4)	Referent	31.4 (25.4, 38.2)	Referent
Region					
Central	208	13.4 (8.2, 21.1)	0.7 (0.4, 1.1)	31.9 (24.7, 40.1)	0.8 (0.6, 1.1)
North	223	20.1 (15.1, 26.2)	Referent	40.8 (33.8, 48.2)	Referent
South	243	12.7 (7.7, 20.1)	0.5 (0.3, 0.9) **	29.8 (23.1, 37.5)	0.7 (0.5, 0.8)*
West	263	22.5 (16.2, 30.4)	1.0 (0.7, 1.1)	29.1 (23.1, 36.0)	0.8 (0.6, 1.1)

¹ **P* < 0.05, ** *P* < 0.01, *** *P* < 0.001. aPRR, adjusted prevalence risk ratio; BLZ\$, Belize dollar.

² Adjusted by area, ethnicity, age, education, income, and region.

TABLE 5

National and regional adjusted geometric means of RBC folate and plasma vitamin B-12 concentrations by sociodemographic characteristics and regions for nonpregnant women of childbearing age, Belize 2011

Characteristic	RBC folate, nmol/L		Vitamin B-12, pmol/L	
	<i>n</i> (unweighted)	Geometric mean (95% CI) ¹	<i>n</i> (unweighted)	Geometric mean (95% CI)
National	937	719 (689, 750)	930	233 (227, 243)
Area				
Rural	559	710 (663, 760)	557	247 (230, 265)
Urban	378	706 (651, 765)	373	245 (227, 265)
Ethnicity				
Maya	139	579 (515, 651) ²	139	214 (188, 243) ³
Mestizo	468	712 (661, 766) ⁴	463	238 (223, 254) ⁵
Black (Creole/Garifuna)	258	755 (666, 788)	256	257 (234, 284)
Other	72	841 (720, 982)	72	280 (249, 314)
Age, y				
15–24	294	671 (618, 729) ⁶	291	249 (230, 271)
25–34	343	709 (653, 769)	340	235 (219, 254)
35–49	300	745 (690, 805)	299	253 (230, 279)
Education, y				
<6	233	736 (673, 804)	232	239 (217, 263)
6–11	354	697 (652, 745)	351	257 (234, 278) ⁷
12	223	700 (643, 762)	220	232 (213, 254)
>12	127	700 (616, 796)	127	257 (233, 284)
Monthly family income, BLZ\$				
Don't know	207	697 (632, 768)	202	234 (212, 259)
699	290	703 (643, 768)	290	246 (224, 271)
700–1399	248	708 (652, 768)	246	249 (228, 271)
>1399	191	724 (656, 800)	191	235 (212, 259)
Health services use				
Private	125	724 (652, 804)	122	278 (246, 315) ⁸
Private/public	100	663 (601, 732)	100	247 (225, 315)
Public	635	728 (683, 776)	631	231 (220, 242)
Other	77	717 (611, 842)	77	230 (201, 265)
Use of folic acid-containing supplements in the last year				
No	538	714 (662, 770)	536	233 (217, 250) ⁹
Yes	399	702 (654, 758)	394	260 (242, 279)
Region				
Central	208	803 (728, 886) ¹⁰	204	254 (231, 281) ¹¹
North	223	701 (628, 782)	221	215 (196, 236)
South	243	682 (616, 755)	242	275 (248, 304)

Characteristic	RBC folate, nmol/L		Vitamin B-12, pmol/L	
	<i>n</i> (unweighted)	Geometric mean (95% CI) ¹	<i>n</i> (unweighted)	Geometric mean (95% CI)
West	263	654 (601, 712)	263	244 (220, 270)

¹ Adjusted for area, ethnicity, age, education, income, health services use, use of folic acid-containing supplements in the last year, and region. All pair-wise comparisons were made.

² Maya vs. Mestizo, $P < 0.002$. Maya vs. Black (Creole/Garifuna), $P < 0.001$. Maya vs. Other, $P < 0.001$.

³ Maya vs. Black (Creole/Garifuna), $P < 0.05$. Maya vs. Other, $P < 0.01$.

⁴ Mestizo vs. Other, $P < 0.05$.

⁵ Mestizo vs. Other, $P < 0.01$.

⁶ 15–19 vs. 35–49 y, $P < 0.01$.

⁷ 6–11 vs. 12 y, $P < 0.05$.

⁸ Private vs. Public, $P < 0.01$.

⁹ No vs. Yes, $P < 0.005$.

¹⁰ Central vs. North, $P < 0.05$. Central vs. South, $P < 0.05$. Central vs. West, $P < 0.001$.

¹¹ Central vs. North, $P < 0.01$. Central vs. South, $P < 0.001$. Central vs. West, $P < 0.05$.